Nemiralisib hydrochloride

Cat. No.: HY-19535

CAS No.: 1254036-77-5

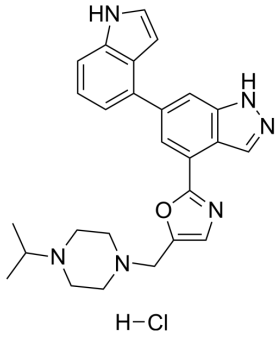
Molecular Formula: $C_{26}H_{29}ClN_6O$

Molecular Weight: 477

Target: PI3K

Pathway: PI3K/Akt/mTOR

Storage: Please store the product under the recommended conditions in the Certificate of Analysis.



BIOLOGICAL ACTIVITY

Description	Nemiralisib hydrochloride (GSK2269557) is a potent and highly selective PI3Kδ inhibitor with a pK_i of 9.9.			
IC_{50} & Target	PI3Kδ 9.9 (pKi)	PI3Kγ 5.2 (pIC_{50})	PI3Kα 5.3 (pIC_{50})	PI3Kβ 5.8 (pIC_{50})
In Vitro	Nemiralisib hydrochloride is highly selective for PI3Kδ, with >1000-fold selectivity over the closely related isoforms PI3Kα (pIC_{50}=5.3), PI3Kβ (pIC_{50}=5.8) and PI3Kγ (pIC_{50}=5.2). Nemiralisib hydrochloride inhibits IFNγ in the peripheral blood mononuclear (PBMC) assay with an pIC_{50} of 9.7[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.			
In Vivo	To assess the suitability of the series for inhaled delivery clearance data in rat microsomes and subsequently in vivo pharmacokinetic data from Sprague Dawley male rats is obtained. Compounds (e.g., Nemiralisib hydrochloride) are administered by the oral or intravenous routes, at a dose level of 3 and 1mg/kg respectively (n=2 rats/route). Nemiralisib hydrochloride is active in a disease relevant brown norway rat acute OVA model of Type 2 helper T-cells (Th2)-driven lung inflammation[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.			

PROTOCOL

Kinase Assay [1]

Inhibition of PI3Kinase enzymatic activity is determined using a homogeneous time resolved fluorescence (HTRF) kit assay format. Reactions are performed in assay buffer containing 50 mM HEPES, pH 7.0, 150 mM NaCl, 10 mM $MgCl_2$, <1 % cholate (w/v), <1 % CHAPS (w/v), 0.05 % sodium azide (w/v) and 1 mM DTT. Enzymes are preincubated with compound, serially diluted 4-fold in 100 % DMSO, for 15 mins prior to reaction initiation upon addition of substrate solution containing ATP at K_m for the specific isoform tested (PI3Kα at 250 μM, PI3Kβ at 400 μM, PI3Kδ at 80 μM and PI3Kγ at 15 μM), PIP2 at either 5 μM (PI3Kδ) or 8 μM (PI3Kα, PI3Kβ and PI3Kγ) and 10 nM biotin-PIP3. Assays are quenched after 60 mins by addition of a quench/detection solution prepared in 50 mM HEPES pH 7.0, 150 mM NaCl, <1 % cholate, <1 % Tween 20, 30 mM EDTA, 40 mM potassium fluoride and 1 mM DTT containing 16.5 nM GRP-1 PH domain, 8.3 nM Streptavidin-APC and 2 nM Europium-anti-GST, and are left for a further 60 mins in the dark to equilibrate prior to reading using a BMG RubyStar plate reader. Ratio data are normalised to high (no compound) and low (no enzyme) controls prior to fitting using a logistical four parameter equation to determine IC_{50}[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Animal Administration [1]	Rats[1] In vivo pharmacokinetics is tested in Sprague Dawley male rats. Compounds (e.g., GSK2269557) are administered discretely by the oral or intravenous routes, at a dose level of 3 and 1 mg/kg respectively (n=2 rats/route). Compounds (e.g., GSK2269557) are formulated as a solution in DMSO:PEG200:water (5:45:50 v/v/v) at a dose volume of 6 (oral) and 2 (intravenous) mL/kg. All animals are serially bled from the tail vein and blood samples collected over a time-course of 0-7 h are submitted to LC-MS/MS analysis for the quantification of the parent compound. The main pharmacokinetic parameters are estimated by non-compartmental analysis. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Down K et al. Optimization of Novel Indazoles as Highly Potent and Selective Inhibitors of Phosphoinositide 3-Kinase δ for the Treatment of Respiratory Disease.

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